Remarks

Amendments to the Claims

The claims have been amended to clarify the claimed method. The basic method is now defined by claim 1, which modifies the prior art systems for making polyhydroxyalkanoates ("PHAs") to be specific to a system for making polymers containing polyhexanoate or copolymers thereof. This requires that the bacteria express a thiolase, reductase and polymerase which can utilize C6 substrates.

Dependent claims define this system modified to make substrate for the thiolase, reductase and polymerase from fatty acids, using either a hydratase or transferase, thereby providing a means for making PHA from cheap sources of substrate. Claim 1 had become inaccurate, confusing three pathways into one. In all cases, however, it is critical to select the thiolase, reductase and polymerase as enzymes that can use C6 substrates.

Support is found as follows. The broad range reductase is active on C_6 substrates (page 14, lines 1-2). The polymerase accepts 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl CoA and the thiolase that accepts acetoacetyl CoA (page 11, lines 9-30). Enzymes for producing substrate such as thiolases specific for 3-ketohexanoyl CoA and reductase acting on 3-ketohexanoyl CoA, and 3-hydroxyhexanoyl CoA (page 12, lines 18-28; page 14, lines 11-14). The D-specific enoyl-CoA hydratase and beta-hydroxyacyl-ACP-coenzyme A transferase genes may be isolated from a bacterium such as *R. eutropha*, *Klebsiella aerogenes*, *P. putida*, and *Aeromonas caviae* (page 5, lines 22-25; claim 10 as originally filed). Fatty acid biosynthetic enzymes which convert acyl

ACP to acyl CoA, such as ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase (Figure 5; lines 8-12, bridging pages 25 and 26).

The Claimed Invention

Polyhydroxyalkanoates (PHAs) are natural, thermoplastic polyesters and can be processed by traditional polymer techniques for use in an enormous variety of applications, including consumer packaging, disposable diaper linings and garbage bags, food and medical products. Several factors are critical for economic biological production of PHAs, including substrate costs, fermentation time, and efficiency of downstream processing. Known biological systems for the production of PHAs containing 3-hydroxy-co-hydroxyhexanoate (3H-co-HH) are inefficient.

The claimed invention is the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in bacteria. Bacteria such as *E. coli* do not normally produce PHAs and have not previously been described to produce 3HH copolymers. PHAs generally are divided into two classes based on the polymer composition: short side-chain PHAs and long side-chain PHAs. Incorporation of monomers from one group into a PHA belonging to the other usually is limited to low levels. Substrate specificities of the PHA polymerases therefore can be generalized as optimal for short side-chains (C_4 and C_5) or medium side-chains (C_8-C_{10}). Based on composition of PHAs synthesized by individual microorganisms, PHA polymerases that incorporate 3-hydroxyhexanoate can be identified. PHA polymerases from *A. caviae*, *C. testosteroni* and *T. pfenigii* are known for incorporating 3-hydroxyhexanoate into the PHA. In contrast, the enzymes from *Paracoccus denitrificans*, *Sphaerotilus natans* and *Rhodococcus sp.*

have a preference for 3-hydroxyvalerate. The PHA polymerases from the latter organisms are useful in making PHB-co-HH copolymers, due to their preference for C_5 over C_4.

A broad substrate range reductase and a polymerase that accepts more than one type of substrate can be used to synthesize polyhydroxybutyrate-co-polyhydroxyhexanoate. One can do this in a more cost effective manner by incorporating enzyme that allows the organism to utilize cheap feedstocks, to produce the substrate for the reductase and polymerase. The bacteria is genetically engineered to produce the co-monomer 3-hydroxyhexanoic acid from feedstocks such as butyrate or butanol, or directly from glucose using enzymes which can channel cellular intermediates to butyryl-CoA. Enzyme activities desirable for conversion of metabolic intermediates into *R*-3-hydroxyhexanoyl CoA, include butyryl CoA dehydrogenase activity and acyl CoA:ACP transferase activities. The latter conversion is catalyzed either by a single protein or by a combination of thioesterase and acyl CoA synthase activities. The flux of normal cellular metabolites to 3-hydroxyhexanoate is redirected via one or more of three different pathways. These three pathways generate 3-hydroxyhexanoate, either (1) using a butyrate fermentation pathway from *Clostridium acetobutylicium*, (2) using fatty acid biosynthetic enzymes from *E. coli*, or (3) using the fatty acid oxidation complex from *Pseudomonas putida*. In a preferred embodiment, *E. coli* is engineered to synthesize PHBH from either inexpensive carbohydrate feedstocks such as glucose, sucrose, xylose and lactose or mixtures of such carbohydrates and fatty acids as the only carbon source by introducing genes encoding enzymes that convert cellular metabolites to 3-hydroxyhexanoyl CoA. It is crucial that the expression of all the genes involved in the pathway be adequate for efficient PHA synthesis in recombinant *E. coli* strains.

A example of a biosynthetic pathway that results in *R*-3-hydroxyhexanoyl CoA formation involves the elongation of butyryl CoA to 3-ketohexanoyl CoA which can subsequently be reduced to the monomer precursor, as shown in Figure 4. Butyryl CoA is formed by butyrate fermenting organisms such as *C. acetobutylicum* in a four step pathway from acetyl CoA. Elongation of butyryl CoA to 3-ketohexanoyl CoA is catalyzed by a thiolase. The complete pathway thus involves (1) the PHB biosynthetic thiolase, (2) the three enzymes from *C. acetobutylicum* that form butyryl CoA, (3) a second thiolase, specific for 3-ketohexanoyl CoA, (4) a reductase specific for this substrate, and (5) a PHB polymerase that accepts both 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl CoA.

In *P. putida*, monomers for PHA biosynthesis are derived from the fatty acid oxidation pathway when alkanes or oxidized alkanes are provided as carbon and energy source. The intermediate in this pathway that is channeled to PHA biosynthesis is *S*-3-hydroxyacyl CoA (preferentially C_8 and C_{10}) which undergoes epimerization by the FaoAB complex to the *R*-isomer. The combined action of epimerase and PHA polymerase provides C_6 to C_{14} monomers for PHA. Consequently, a combination of this epimerase and a 3-hydroxyhexanoyl CoA accepting PHA polymerase provides the biosynthetic capability to synthesize PHBH from fatty acids in transgenic organisms, as shown by Figure 5. Mixtures of fatty acids and carbohydrates that are useful feedstocks for fermentative production as the 3HB monomer can be derived from acetyl CoA, where the 3HH component is from fatty acids.

P. putida and *P. aeruginosa* synthesize PHAs composed of medium-chain length 3-hydroxy fatty acids when grown on sugars. The predominant monomer in these PHAs is 3-

hydoxydecanoate. A similar pathway can be engineered for the synthesis of PHBH in recombinant microorganisms such as *E. coli, R. Eutropha* and *P. putida*, as shown by Figure 6. Besides a polymerase that accepts the 3-hydoxybutyryl CoA and 3-hydroxyhexanoyl CoA precursors, an enzymatic activity that converts 3-hydroxyacyl ACP into 3-hydroxyacyl CoA or 3-ketoacyl ACP into 3-ketoacyl CoA is required. Deregulation of fatty acid biosynthesis and increased activity of this pathway subsequently provides the substrate for PHBH formation. The critical enzymatic activity in this pathway is the conversion of the 3-hydroxyacyl ACP to the CoA derivative. Thioesterases and acyl CoA synthases can accomplish this step. Alternatively, acyl ACP:CoA transferase can be used to facilitate this step in the PHA pathway.

Claim 6 defines the method wherein the phbC polymerase gene encodes a PHA polymerase enzyme that incorporates C6 substrates and is incorporated into the bacterial chromosome. Claim 7 defines the method wherein the phbC polymerase gene is from *Aeromonas caviae, Comamonas testosteroni, Thiocapsia pfenigii, Chromatium vinosum, Bacillus cereus, Nocardia carolina, Nocardia salmonicolor, Rhodococcus ruber, Rhodococcus rhodocrous,* or *Rhodospirilum rubrum.* Defining the enzymes by their source also provides information as to their substrate specificity, since as discussed above, the specificity and products naturally made by these bacteria are known.

Claim 10 defines the system of claim 1 further comprising a gene encoding a ß-hydroxyacyl-ACP-coenzyme A transferase.

Claim 14 defines the bacteria as an *E. coli.*

Claim 15 defines the source of the polymerase accepting 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl CoA.

Claim 16 further defines the bacteria as expressing a D-specific enoyl-CoA hydratase.

Claim 17 defines the bacteria as one expressing a PHB biosynthetic thiolase, the three enzymes from *C. acetobutylicum* that form butyryl CoA, the thiolase specific for 3-ketohexanoyl CoA, reductase specific for 3-ketohexanoyl, and PHB polymerase that accepts both 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl CoA.

Claim 18 defines the *E. coli* as expressing one or more fatty acid biosynthetic enzymes. Claim 19 requires the fatty acid biosythetic enzymes to convert acyl ACP to acyl CoA. Claim 20 defines these enzymes as ACP-CoA transacylase, acyl ACP thioesterase, and/or acyl CoA synthase. Claim 21 defines the enzymes as acyl ACP thioesterase and acyl CoA synthase.

Rejection Under 35 U.S.C. § 112, enablement

Claims 1, 6, 7, 10, and 14-21 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection.

The Legal Standard

The test of enablement is whether one of ordinary skill in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *United States v. Telectronics, Inc.*, 857 F.2d 778, 8 U.S.P.Q.2d 1217 (Fed. Cir. 1988); *In re Stephens*, 529 F.2d 1343, 199 U.S.P.Q. 659 (C.C.P.A. 1976). A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929

F.2d 660, 661, 18 U.S.P.Q.2d 13321, 1332 (Fed. Cir. 1991); *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 3 U.S.P.Q.2d 1737 (Fed. Cir. 1987).

The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt,* 537 F.2d 498, 190 U.S.P.Q. 214 (C.C.P.A. 1976). Whether undue experimentation is needed is not based upon a single factor; it is a conclusion reached by weighing many factors. These factors have been summarized in *In re Wands,* 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988) and include, but are not limited to:

(1) The quantity of experimentation necessary (time and expense);

(2) The amount of direction or guidance presented;

(3) The presence or absence of working examples of the invention;

(4) The nature of the invention;

(5) The state of the prior art;

(6) The relative skill of those in the art;

(7) The predictability or unpredictability of the art; and

(8) The breadth of the claims.

The M.P.E.P. explains that "[i]t is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above factors while ignoring one or more of the others." Thus, a conclusion of nonenablement must be based on the evidence as a whole, as related to each of these factors (see M.P.E.P. § 2164.01 (a)).

In *Enzo Biochem*, the Federal Circuit held that that the written description requirement can be met by a functional description of claimed materials, if coupled with a known or disclosed

correlation between function and structure. *Enzo Biochem, Inc., v. Gen-Probe, Inc.*, 296 F.3d 1316, 63 U.S.P.Q.2d 1609 (Fed. Cir.2002) ("*Enzo II*"). The Federal Circuit adopted provisions from the Guidelines issued by the U.S. Patent and Trademark Office that state that the written description requirement can be met by a functional description of claimed materials, if coupled with a known or disclosed correlation between function and structure. The court found that the written description requirement was met when, in the knowledge of the art, the disclosed function is sufficiently correlated to a particular, known structure.

In its most recent decision regarding issues of written description and enablement as defined by 35 U.S.C. § 112, *Amgen, Inc. v. Hoechst Marion Roussel, Inc. and Transkaryotic Therapies, Inc.*, 314 F.3d 1313 (Fed. Cir. 2003), the Court of Appeals for the Federal Circuit continued in the manner of *Enzo II* and applied a broad interpretation of the types of disclosures that comply with the written description requirement. Similarly, in *Amgen*, the Federal Circuit adopted a broad interpretation of the types of disclosures that meet the enablement requirement.

The Federal Circuit stated "because the claim terms at issue here are not new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend. Instead, the claims of Amgen's patents refer to types of cells that can be used to produce recombinant human EPO. [...] This difference alone distinguishes *Eli Lilly*, because when used, as here, merely to identify types of cells (instead of undescribed, previously unknown DNA sequences), the words 'vertebrate' and 'mammalian' readily 'convey[] distinguishing information concerning [their] identity' such that one of ordinary skill in the art could 'visualize or recognize the identity of

members of the genus." *Amgen*, 314 F.3d at 1332, citing *Eli Lilly*, 119 F.3d at 1567, 1568, 43 U.S.P.Q.2d at 1406.

The Federal Circuit stated that Amgen's invention was not "the location of the control sequences and EPO DNA in relation to the cell, but rather the production of human EPO using those sequences." *Id.* The court held that the claims were enabled based on two rationales. First, in response to TKT's argument that the claims were not enabled since the specification did not teach a method for making the EPO using endogenous DNA, the court explained, "The specification's failure to disclose the later-developed endogenous activation technology cannot invalidate the patent." *Id.* at 1335, citing *Amgen*, 126 F. Supp. 2d at 160, 57 U.S.P.Q.2d at 516. Second, the court referred to earlier cases for the rule that "the law makes clear that the specification need teach only one mode of making and using a claimed composition." *Id.*, citing *Johns Hopkins Univ. v. Cellpro, Inc.*, 152 F.3d 1342, 1361, 47 U.S.P.Q.2d 1705, 1719 (Fed. Cir. 1998); *Engel Indus. Inc. v. Lockformer Co.*, 946 F.2d 1528, 1533, 20 U.S.P.Q.2d 1300, 1304 (Fed. Cir. 1991). The court also held that the '422 patent was enabled since the specification "described and enabled at least one way of obtaining EPO purified from mammalian cells in culture." *Id.*

Analysis

The issue is whether or not one skilled in the art would know what genes are required to make the claimed bacteria, and whether or not it would require undue experimentation to make and use the claimed bacteria. As demonstrated by the actual working examples in the specification, those skilled in the art would know what enzymes are required and would either be

able to use those publicly available, described in the literature, or obtained without undue experimentation using the information provided by Applicants.

There appears to be confusion as to whether or not one requires the sequence encoding the enzymes. Unlike in some systems such as eukaryotic cells, genes have been identified by their activities and transferred into bacteria, either in plasmids or incorporated into the genome, without the sequence being known, for decades. What is important is to know a source for the genes, and what the genes must encode.

As apparent from the discussion of the recent Federal Circuit decisions, this requirement may be different if one is claiming the genes, but in this application, applicants are claiming methods of making a polymer using new combinations of known materials, very similar to the fact situation in *Amgen v. Hoescht, et al.*, 314 F.3d 1313 (Fed. Cir. 2003).

The claims require several enzymes:

(1) a phbA thiolase gene encoding an enzyme that converts butyryl-CoA and acetyl CoA to beta-ketohexanoyl-CoA,

(2) a phbB reductase gene that encodes an enzyme that converts beta-ketohexanoyl-CoA to beta-hydroxyhexanoyl-CoA,

(3) a phbC polymerase gene that encodes an enzyme that polymerizes 3-hydroxybutyryl CoA, and optionally, one of the following:

(4) a D-specific enoyl-CoA hydratase,

(5) ß-hydroxyacyl-ACP-coenzyme A transferase, and

(6) fatty acid biosythetic enzymes to convert acyl ACP to acyl CoA, specifically ACP-CoA transacylase, acyl ACP thioesterase, and/or acyl CoA synthase.

Although there is no requirement for examples, the Applicants have provided numerous working examples showing how one of ordinary skill would obtain the genes to be transferred to recipient cells for expression. Example 4, at pages 23 and 24 of the specification, clearly illustrates a method of isolating the *pha*J gene encoding an enoyl-CoA hydratase from chromosomal DNA prepared from an *A. caviae* strain. Specific primers were used in polymerase chain reactions to amplify the gene. The sequence of the primers are shown on page 24, lines 9-15. Plasmids were constructed and subsequently used to transform recipient *E. coli* cells. Once transformed, the cells express the hydratase gene as evidenced by the production of polyhydroxybutyrate-co-polyhydroxyhexanoate containing 2.6% HH comonomer. This copolymer was analyzed *via* gas chromatography as shown at page 24, lines 24-25.

The ß-hydroxyacyl-ACP-coenzyme A transferase gene encodes an enzyme that converts 3-hydroxyacyl ACP to the CoA derivative. This step in the polyhydroxyalkanoate pathway is facilitated by acyl ACP:CoA transferase activity. Genes that encode this enzyme can easily be identified in bacteria that produce polyhydroxyalkanoates from oxidized carbon sources, such as carbohydrates (see page 15, lines 18-23, of the specification). Methods used to isolate genetic sequences are well known. These methods can be applied to the isolation of any of the genes used in the claimed methods. Example 1, on page 19 of the specification, discusses a routine method used for the isolation of specific genes. As in Example 4, discussed above, Example 1 illustrates the amplification and isolation of the *pha*C gene, encoding the polyhydroxyalkanoate

("PHA") polymerase enzyme, and the phaB gene, encoding the PHA reductase enzyme, from *N. salmonicolor* chromosomal DNA. Example 3 further shows, using the same methods of Examples 1 and 4, how one would isolate the *hbd*, *crt*, and *bdh* genes from *C. acetobutylicum* (see page 22 of the specification).

The identification of genes encoding enzymes that convert acyl ACP to acyl CoA is presented in Figure 10 as a screen that makes use of the very user friendly *lux* system of *Vibrio fischeri*. One merely assays for light generation that results from the induction of the transgenic lux system. Such light generation implicates ACP::CoA transferase activity that is present in the system.

The foregoing primer and PCR methods, combined with the user friendly screen for genes encoding enzymes that convert acyl ACP to acyl CoA, are well within the skill of one in the art and would not require undue experimentation.

The enzymes are defined by their substrate specificity. As discussed at page 5, lines 25-27, of the specification, "the genes are preferably selected on the basis of the substrate specificity of their encoded enzymes being beneficial for the production of the 3HH polymers." The substrate, in the presence of its cognate active enzyme, will be readily converted into product (i.e. the substrate for another enzyme). Based upon the specification, one of ordinary skill in the art will appreciate that the presence, or production, of end-product (i.e. polyhydroxybutyrate-co-polyhydroxyhexanoate) is easily measured and characterized using methods well known in the art. Page 18, line 29, to page 19, line 10, describes in detail the method of using gas chromatography to analyze the polyhydroxyalkanoate produced from the claimed methods. Gas

chromatography was used in each of Examples 2, 4, and 5 to analyze the final content of the copolymer produced (1% HH comonomer, 2.6% HH comonomer, and 1.2% HH comonomer, respectively).

The ease in which one may assay for the final copolymer product, as described in the preceding paragraph, supports enablement for use of the specific enzymes of claims 14-17. Each of a broad range reductase that is active on C_6 substrates, a polymerase that accepts 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl CoA, a thiolase that accepts acetoacetyl CoA, a thiolase specific for 3-ketohexanoyl CoA, and a reductase active on 3-ketohexanoyl CoA and 3-hydroxyhexanoyl CoA (claim 17), can be easily assayed for activity by gas chromatographic analysis. The presence of the copolymer end product will tell the investigator whether or not the overall process of providing proper substrate for enzyme catalysis at each step in the copolymer synthetic pathway is successful. The genes encoding each of these enzymes can be isolated by using common PCR techniques discussed above, or obtained from the ATCC, or through catalogs or as described in the literature. All of the claimed enzymes are known and characterized.

The discovery that a broad substrate range reductase and a polymerase that accepts more than one type of substrate could be used in a single pathway dedicated to the synthesis of polyhydroxybutyrate-co-polyhydroxyhexanoate, was a completely unexpected result. Such a combination of enzymes resulted in PHBH copolymer containing 1.0% HH co-monomer in Example 2. This pathway is predicated upon efficient substrate/enzyme interactions and reactions.

The fatty acid biosynthetic enzymes of claims 18-21 make use of distinctly different substrates than those of the other enzymes. The fatty acid biosynthesis pathway requires an enzyme activity that converts acyl ACP into acyl CoA, a reaction catalyzed by an ACP/CoA transacylase or by the combined action of an acyl ACP thioesterase and acyl CoA synthase. It is clear from the specification at page 25, line 20, to page 26, line 12, that the *lux* system of *V. fischeri* is an ideal tool for easily screening genetic sequences encoding the desired activity that converts acyl ACP into acyl CoA. Such a screening tool enables the identification of ACP/CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase enzymatic activity. Once the activity is identified, the corresponding genetic sequence is easily isolated from the plasmid initially used to transform *V. fischeri*. The sequence is subsequently amplified using well known PCR techniques.

Rejection Under 35 U.S.C. § 112, written description

Claims 1, 6, 7, 10, and 14-21 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection.

The Legal Standard

The law has long allowed an Applicant to claim all that he is entitled to, not forcing him to limit his claims to a specific example, if other means for achieving the same step would be known to those skilled in the art and not require undue experimentation. That is clearly the case here.

The most recent articulation of the requirement under 35 U.S.C. 112, for written description, was made by the Court of Appeals for the Federal Circuit in its decision in *Amgen Inc. v. Hoechst Marion Roussel, Inc and Transkaryotic Therapies, Inc*, 314 F3d 1313, 65 USPQ2d 1385 (Fed. Cir. 2003), discussed above.

> "Section 112 of the patent statute describes what must be contained in the patent specification. Among other things, it must contain "a written description of the invention, and of the manner and process of making and using it . . . [such] as to enable any person of ordinary skill in the art to which it pertains . . . to make and use the same . . . ." 35 U.S.C. § 112 ¶ 1. Thus, this statutory language mandates satisfaction of two separate and independent requirements: an Applicant must both describe the claimed invention adequately and enable its reproduction and use. *See Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991)."
>
> "The purpose of the written description requirement is to prevent an Applicant from later asserting that he invented that which he did not; the Applicant for a patent is therefore required to "recount his invention in such detail that his future claims can be determined to be encompassed within his original creation." Id. at 1561, 19 USPQ2d at 1115 (citation omitted). **Satisfaction of this requirement is measured by the understanding of the ordinarily skilled artisan.** *Lockwood v. Am. Airlines, Inc.* , 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997) ("The description must clearly allow persons of ordinary

skill in the art to recognize that [the inventor] invented what is claimed.").

"Compliance with the written description requirement is essentially a fact-based inquiry that will 'necessarily vary depending on the nature of the invention claimed.'" *Enzo Biochem v. Gen-Probe, Inc.*, 296 F.3d 1316, 1324, 63 USPQ2d 1609, 1613 (Fed. Cir. 2002) (citation omitted)."

"Indeed, the district court's reasoned conclusion that the specification's description of producing the claimed EPO in two species of vertebrate or mammalian cells adequately supports claims covering EPO made using the genus vertebrate or mammalian cells, renders *Eli Lilly* listless in this case. *Amgen*, 126 F. Supp. 2d at 149, 57 USPQ2d at 1507."

Applicants are claiming a method for the production of a polymer in bacteria. Therefore, what is actually being claimed are new combinations of known materials. Each of the reagents or materials used in the claimed methods can be ordered from the American Type Culture Collection (ATCC), or obtained from information disclosed in scientific publications as described in the specification. For example, PHA synthase genes have been isolated from *Aeromonas caviae*, as disclosed in Fukui & Doi; *Rhodospirillum rubrum*, as disclosed in U.S. Patent No. 5,849,894; *Rhodococcus ruber*, as disclosed in Pieper & Steinbuechel; and *Nocardia corallina*, as disclosed in Hall *et. al.* Each of the foregoing four references is cited at page 10, line 28, to page 11, line 2. In their response and amendment mailed on March 10, 2003, the Applicants submitted published sequence listings from the National Center for Biotechnology Information. Included were *phbB* amino acid and nucleotide sequences from *Asospirillum*

brasilense and *Pseudomonas putida*; *phb*A amino acid and nucleotide sequences from *Pseudomonas putida*; and *phb*C amino acid and nucleotide sequences from *Pseudomonas putida*, *Rhodobacter sphaeroides*, *Azorhizobium caulinodans*, *A. eutrophus*, and *Pseudomonas sp.* Furthermore, at page 14 of the specification, fatty acid oxidation gene subunits were isolated from *P. putida* KT 2442 and expressed to show epimerase activity.

The enzymes are defined by their substrate specificity. As discussed at page 5, lines 25-27, of the specification, "the genes are preferably selected on the basis of the substrate specificity of their encoded enzymes being beneficial for the production of the 3HH polymers." The substrate, in the presence of its cognate active enzyme, will be readily converted into product (i.e. the substrate for another enzyme).

In addition to those nucleic acid sequences defined as specific *phb*A, *phb*B, PHB, *phb*C, and *pha*J genes in the specification, the primer and/or oligonucleotide sequences used to hybridize to, and isolate, those sequences can be used to isolate other genes encoding the claimed enzymes. This is routine to those skilled in the art. Methods include "colony blotting using the corresponding PCR products as probes," as disclosed at page 12, lines 13-15; colony blot hybridization using full length genes as probes, as disclosed at page 20, lines 13-16; Southern blotting, wherein "DNA fragments containing the phbB and phbC genes from *N. salmonicolor* were identified in genomic digests by Southern blotting using the corresponding PCR products as probes," as disclosed at page 12, lines 11-13; and PCR isolation/amplification, wherein the primers that are based on the nucleotide sequence of the phaC gene from *Rhodococcus ruber* and conserved regions in the N- and C- terminal ends of known acetoacetyl CoA dehydrogenases are

used to isolate the corresponding PHB biosynthetic enzymes from *N. salmonicolor*, as described at page 12, lines 4-11. The methods in which one of ordinary skill in the art would use to isolate the claimed genes lie at the very heart of defining the structural nature of each gene. The structural feature common to the members of the claimed genus of pha genes have already been determined based upon the hydrogen bonding arrangement of the primers and oligos used to isolate such sequences. The structures of the claimed genes are clearly limited based, in part, on the requirement for them to be complementary to the primers and/or oligos disclosed, for example, in Examples 1, 3, and 4. Once isolated, construction of gene expression cassettes and transformative plasmids, as described in the specification, are easily produced. Plasmids are introduced *via* known methods, such as transformation.

Not only can the genes be readily obtained from various collections or personally from other scientific investigators, but much data had already been accumulated on the specific enzymatic reactions that each of the gene-encoded enzymes catalyze. This information is explicitly provided in the references cited on page 11 of the specification. For example, Ploux *et al.* disclose that the reductase enzyme from *Z. ramigera* is most active with acetoacetyl CoA, whereas 3-ketovaleryl CoA and 3-ketohexanoyl CoA were also substrates for the enzyme. Haywood *et al.* disclose that *R. eutropha* has two 3-ketothiolases which have higher activity toward acetoacetyl CoA substrates than 3-ketovaleryl CoA substrates.

The fatty acid biosynthetic enzymes are also defined by their substrates. Many are known, cloned and well characterized. Homologous genes are readily isolated from bacteria such as *R. eutropha*, *A. latis*, *C. testosteroni*, *P. denitrificans*, *R. ruber*, and other PHA and non-

PHA producers using the same methods to identify the *fao*AB (fatty acid oxidation) genes in *P. putida* KT2442. This is explicitly stated at lines 30-3, bridging pages 14 and 15 of the specification. Furthermore, epimerase activity had been detected in the fatty acid oxidation complexes of *E. coli* and *P. fragi*. As disclosed at page 14, lines 21-26, each of the *Fao*AB complex subunits were cloned and expressed to show the substrate specificity of components of the PHA pathway in *P. putida*.

Claim Objections

Claims 14-21 were objected to for depending from a canceled claim. Applicants respectfully traverse this objection to the extent that it is applied to the claims as amended.

Claims 14-18 have been amended to depend from claim 1. Therefore the amended claims depend from a pending claim.

Allowance of claims 1, 6, 7, 10, and 14-21, as amended, is respectfully solicited.

Respectfully submitted,

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Certificate of Facsimile Transmission

I hereby certify that this Amendment and Response to Office Action, and any documents referred to as attached therein are being facsimile transmitted on this date, February 3, 2004, to the Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450.

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Date: February 3, 2004

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